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(54) Title: IMPROVEMENTS RELATING TO THE SPECIFICITY OF GENE EXPRESSION

(57) Abstract

This invention seeks to improve the specificity of gene expression by targeting a specific expression site of a target gene. There is thus provided a chimaeric gene which comprises a promoter which expresses in more than one region of the organism to be affected. The promoter is linked to an agent which affects the functioning of an endogenous gene in the plant which is also expressed in more than one region of the plant. The promoter and agent are selected so that there is an overlap in their expression sites at one or more desired locations. This overlap site(s) gives increased specificity and targeting of gene expression.

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IMPROVEMENTS RELATING TO THE SPECIFICITY OF GENE EXPRESSION

This invention relates to a method for increasing the specificity of gene expression. In particular, the invention relates to the use of a chimaeric gene to generate a highly specific targeting effect as a mode of providing, for example but not exclusively, a targeted resistance to disease-causing agents in plants.

For the purpose of this invention it is useful to describe in a simplified manner how a gene is constructed and how it functions (see Figure 1 which shows the structure of a gene, which gene may also be a chimaeric gene). A gene can be considered to consist of three components (the numbers refer to the numbers in Figure 1); 1. a promoter (P) which determines when and where a coding sequence is transcribed, a coding sequence (CS) for the production of a protein and a 3' regulatory sequence (3') that can sometimes also affect the transcription of the coding sequence. The 3' regulatory sequence is commonly known as a terminator. 2. A gene is expressed when the promoter permits the transcription and processing (10) of a working copy of the coding sequence to produce a messenger RNA (mRNA). 3. The mRNA is then translated (11) to give a protein product. 4. The protein product can then interact with a substrate or another protein or a regulatory sequence to cause an effect (E).

However, it should be noted that regulation of the expression of a gene can be affected at each stage of the expression process described above. Factors can act on the

promoter, on the transcription machinery to produce the mRNA and on processes that modify the mRNA or affect its stability. Factors can also act on the translation of the protein and on the turnover of the protein within the cell. Other factors can influence how the protein interacts with other components and achieves its effect.

To explain the inventive concept, consider the following genes, gene 1 and gene 2 in a plant. Gene 1 is active in tissues A and B of a plant, whilst gene 2 is active in regions B and C of a plant. The activities of the two genes overlap in region B and this can be described visually as in Figure 2, which is in the form of a Venn diagram. From this Figure it is apparent that the site of joint expression is more limited or, in other words, more specific than the sites of expression of either gene on their own.

Thus in this invention, in respect of plants, the chimaeric gene contains a promoter which expresses in more than one region of the plant. The promoter is linked to an agent. The agent will affect the functioning of an endogenous gene which is also expressed in more than one region of the plant. The areas in which the promoter and the endogenous gene to be affected are active are not identical but they do overlap at one or more desired locations. When the chimaeric gene is transferred into a plant, the agent will only have an effect on the target endogenous gene at the location(s) of overlap.

There are several ways to exemplify the invention, of which increased tolerance or resistance to plant parasitic nematodes is a practical example. Although we have used cell

disruption and male sterility as examples, the system can also be used for the enhancement of a gene at a particular site.

Several different mechanisms have been proposed to obtain cell specific disruption. The simplest method utilises a chimaeric gene comprising a promoter specific to the targeted tissue linked to a disruptive system. Even specific promoters, however, may express to a lesser degree in tissues other than those targeted, which is sometimes undesirable.

Other applications have attempted to circumvent this problem by utilising two constructs, the first construct containing a chimaeric gene comprising a tissue specific promoter linked to a cell necrosis system (e.g. barnase) and the second construct containing a chimaeric gene comprising a promoter active in regions other than the one targeted, the promoter being linked to a protectant (e.g. barstar) which inactivates the necrosis system. The necrosis in tissues other than the one desired is therefore suppressed by the protectant (see International Patent Application Nos. WO92/21757 (Plant Genetic Systems N.V.) and WO 93/10251 (Mogen International N.V.)).

This invention can provide, for example, a highly specific cell disruption system using a single construct. Other cell regulation systems to which the invention is applicable will be known to the skilled man.

It is an object of the invention to provide a site specific expression system or targeting system which is generally applicable to any organism which has genes expressed

in different regions but where their sphere of expression overlaps.

It is also an object of the invention to achieve increased specificity using only one construct comprising a chimaeric gene comprising two or more nucleic acid sequences, which construct is designed to interact with an endogenous gene in an organism.

The present invention provides a method of improving the specificity of gene regulation in a transformed organism, the method comprising the steps of identifying an endogenous target gene in an organism, determining the location of more than one expression sites of the target gene, creating a chimaeric gene comprising a promoter which causes gene expression at at least two expression sites in an organism, including expression at one of the expression sites of the target gene, and an agent being a nucleic acid sequence which regulates expression of the target gene or a product thereof, stably incorporating the chimaeric gene into a cell of the organism by genetic transformation, and regenerating an organism from the transformed cell, which transformed organism contains the chimaeric gene, the expression of which gene in the organism causes the target gene or a product thereof to be regulated at at least one specific expression site in the transformed organism or progeny thereof.

Preferably the nucleic acid sequence is a coding or a non-coding sequence.

Preferably the expression of the target gene can be up regulated or down regulated.

Preferably the organism is a plant, whereby the transformed plant or propagules thereof contain the chimaeric gene.

The mechanism whereby the agent acts on a gene could belong to any one of the following. The list should not be considered to be exclusive.

1. antisense.
2. cosuppression.
3. inhibition or activation of the promoter of a target gene.
4. inhibition or activation of transcription.
5. alteration of messenger RNA stability or degradation of mRNA.
6. inhibition or activation of translation.
7. inhibition or activation of a protein
8. alteration of protein turnover.
9. acting as a cofactor.
10. alteration of protein-protein interactions.
11. alteration of the flux through a biochemical pathway.

Some examples of these mechanisms are discussed briefly below. It should be borne in mind that the particular mechanism to be selected to achieve gene regulation will also require a certain level of site specific expression in order to be effective in the inventive concept.

Down regulation may advantageously be achieved by the agent of the chimaeric gene being a nucleic acid sequence which is the antisense orientation of the whole or part of the promoter or a coding or non-coding sequence of the target gene.

Alternatively, down regulation may be achieved by cosuppression of the promoter or coding or non-coding sequence of the targ t gene.

Up regulation of the target gene may be achieved, for example, by introducing an activator of the promoter of the target gene.

Combinations of these technologies may also be used.

Other suitable methods of regulating gene expression of the target gene will be known to those skilled in the art.

The agent of the chimaeric gene may comprise one or more nucleic acid sequences, each of which sequences, when expressed, carries out a particular function. There may thus be obtained specificity of expression of more than one endogenous gene using only one promoter.

A construct can contain two separate chimaeric genes as expression cassettes, each chimaeric gene comprising a promoter, a coding sequence for an agent and a terminator. Each chimaeric gene acts on a different endogenous gene, which gene may be expressed at the same target site or a different site. There is thus the possibility of knocking out or increasing several components or target genes in a sequence of events, such as a particular biosynthesis chain, over a period of time. In other words, a temporal chain of events can be effected. Alternatively, each chimaeric gene could be transferred into the organism in two separate constructs, each construct containing one expression cassette, i.e. one chimaeric gene.

The nucleic acid sequence may be a DNA sequence or an RNA sequence.

The promoter of the chimaeric gene may be expressed in more than one overlapping expression site of the endogenous gene.

Advantageously the promoter is a nematode-inducible promoter, such as the promoter known herein as the KNT1 promoter or the RB7 promoter. Other promoters which are caused to express by other agents acting on them at other attacked or growth sites may be utilised depending on the site specific expression regulation to be achieved.

Advantageously the nucleic acid sequence is the or a part of the antisense sequence of the RB7 or KNT1 promoter or coding sequence thereof.

Alternatively the nucleic acid sequence may be a ribozyme or a targeted RNase to degrade a messenger RNA in order to effect, for example, mechanism 5 of the list of mechanisms above. Also, specific RNA's can be stabilised or destabilised by specific nutrients, e.g. iron in the case of the mRNA for the cell surface protein receptor for transferring; or ligands, hormones and translation products, e.g. the effect of tubulin protein dimer on tubulin mRNA. Selection of nutrients, ligands, hormones or translation products expressed or required at certain locations is desirable for the inventive concept.

An example of an activator of transcription is the heat shock factor of *Drosophila* which encodes a protein free in the cell. Upon heat shock, the heat shock gene factor binds to the promoter of the heat shock protein HSP70 and leads to increased

transcription. Heat shock proteins are found in bacteria, animals and plants. Activators suitable for use in the inventive concept which are site specific can be selected by the skilled man to achieve mechanism 4.

Suitable cofactors for effecting mechanism 9 in an organism include vitamins such as pantothenic acid and vitamin B6.

Finally, mechanism 10 could be effected by introducing, for example, the protein cAMP-dependent protein kinase into an organism. The cAMP-dependent protein kinase acts upon the the enzyme glycogen synthase by phosphorylating it. The glycogen synthase is turned into a less active form and glycogen synthesis is inhibited.

The invention may also utilise a number of constructs, each promoter-gene fragment of the chimaeric gene of each construct having an overlap at the same target expression site as each of the other promoter-gene fragments, so that there is provided multiple overlap at the selected single expression site to further enhance the specificity of the system. The other expression site of the chimaeric gene may be the same as or different from the other expression sites of the other promoter-gene fragments.

In order that the invention may be easily understood and readily carried into effect, reference will now be made, by way of example, to the following figure and example in which:

Figure 2 shows the overlap of the expression sites of the two separate genes when they are in the same plant,

Figure 3 shows the map for vector pATC37010 used to transform plants in accordance with the invention,

Figure 4 shows the map for vector pATC37003, a control vector used to transform plants, and

Figure 5 shows the map for pATC into which SEQ.ID.No.5 and SEQ.ID.No.6 were ligated to produce the vectors pATC37010 and pATC37003 of Figures 3 and 4 respectively.

Cell specific disruption at site B in Figure 2 can be achieved as follows. The promoter which regulates the specificity of expression from gene 1 can be linked to a region which will disrupt the activity of gene 2 in a construct. When the construct is introduced into plants, the agent disruptive of gene 2 will be expressed in regions A and B. There will be no effect at site A because gene 2 is not active here. There will be no effect at site C because the promoter of the construct is not active there and hence no disruptive agent for gene 2 is produced. There will be disruption of gene 2 at site B as the disruptive agent for gene 2 is present and gene 2 is also active.

EXAMPLE 1

Engineering increased tolerance or resistance to plant parasitic nematodes using Venn constructs.

Plant parasitic nematodes such as the root knot nematodes and cyst nematodes cause 7 to 14% losses in crop yield world wide. The nematodes act by penetrating plant roots and generating unique feeding sites through which they derive their

nutrients. The feeding sites are altered plant cells, either giant multinucleate cells in the case of root knot nematodes, or syncitia of several cells fused together in the case of cyst nematodes. The nematodes become sessile and are totally dependent on the feeding sites for nutrients. Our U.S. Patent No. 5,589,622 describes a general way of making plants resistant by linking feeding cell specific promoters to cell death or cell disruption systems to disrupt the feeding cells. The feeding cells are impaired in their function so the nematode starves or has a reduced food supply and is unable or less able to grow and to produce offspring. This method is an example of the simplest method of cell specific disruption described above. Other patents which utilise this principle are those which create sterility in a plant, for example, International Patent Application No. WO 89/10396 (Plant Genetic Systems N.V.)

Promoter KNT1 which is expressed in feeding cells, root tips and to a lesser extent in other meristems was identified. Other workers have identified a gene, RB7 expressed in roots and giant cells (Conkling *et al* 1990, Opperman *et al* 1993). Our studies with the RB7 promoter linked to the marker gene GUS suggest that the RB7 gene is expressed in the body of the root and not at the root tip. A Venn construct containing the promoter to KNT1 linked to a partial antisense sequence of RB7 coding sequence and a nos terminator in a pBIN19 (Bevan, M. 1984) derived plant transformation vector containing *Agrobacterium tumefaciens* C58 was made. The construct was labelled pBIN05002 and was deposited by Advanced Technologies

(Cambridge) Ltd of 210 Cambridge Science Park, Cambridge CB4 4WA, England under the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the purposes of Patent Procedure at the National Collection of Industrial and Marine Bacteria (NCIMB), 23 St. Machar Street, Aberdeen, Scotland on 20th March 1997 under accession number NCIMB 40871. Tobacco plants cv Heavy Western were transformed with this construct using Agrobacterium mediated plant transformation in accordance with the method of Horsch *et al* (1985).

Regenerated transgenic plants were transferred to the greenhouse. Transgenic plants and non-transgenic controls were each infected with about 100 individuals of the root knot nematode *Meloidogyne javanica*. Eight weeks after infection the number of visible root knots and their size was determined. During this period the initial inoculum had the opportunity to infect, mature and produce a second generation of nematodes which in turn could infect the plant roots and mature.

Eight of the lowest scoring transgenic pBIN05002 plants were grown to seed. Progeny from parent plants were again screened for increased tolerance or resistance to *M. javanica* as described above. In addition to the pBIN05002 plants, progeny from plants transformed with pBIN05101 containing the KNT1 promoter linked to the glucuronidase marker gene (GUS) (Jefferson, R.A. *et al* 1987) and progeny from untransformed plants were included as controls for comparison. pBIN05101 was also deposited at the NCIMB on 20th March 1997 under accession number NCIMB 40870. Progeny from pBIN05002 plant line 32 showed a significant increase in the number of plants with low

gall scores as shown in Table 1. The results are significant in a Chi square analysis.

Table 1

Number of plants in low and high gall score categories for untransformed control plants, control pBIN05101 plants and pBIN05002 test plants.

Low gall score = 0 to 50 galls per plant.

High gall score = 51+ galls per plant.

Tr atment	plants with low gall score	plants with high gall score
Untransformed plants	18	13
pBIN05101 control plants	13	17
pBIN05002 line 32 test plants	25	7

EXAMPLE 2

The overlap principle illustrated in the above example using a cell disruption system for increased specificity in nematode disruption can equally well be carried out in the flower of *Arabidopsis*, for example, or other plants to provide flowers with altered flower pattern or structure, for example, male sterility. This example utilises DNA sequences identified in *Arabidopsis*.

There are four elements of the flower (sepal, carpel, petal and stamen) which are postulated to be under the control of three genes (Coen, E.S. and Meyerowitz, E.M., 1991).

Altering the balance of these genes causes a variation in flower pattern. For example, both the gene *agamous* and *apetala3* must be expressed in the same part of the flower to give rise to the male part of the plant, the stamen. *Agamous* is expressed both in the carpels and stamens, whilst *apetala3* is expressed in both stamens and petals.

In order to make a construct following the overlap principle which is the subject of this invention, we require the promoter from one gene (e.g. *agamous*, active in carpels and stamens) linked to a disruptor of a second gene (e.g. *apetala3*, active in petals and stamens) to effect disruption in only the stamens.

A 435 basepair fragment of the *agamous* promoter was isolated from *Arqabidopsis thaliana* DNA using the polymerase chain reaction with Taq and Taq-extender using the following two primers according to published procedures (Thomas, C., 1996):

Primer 1 (SEQ.ID. No. 1)

ATCGAAGCTT CTAAATGTAC TGAAAAGAAA CA

Primer 2 (SEQ.ID. No.2)

ACTGGGATCC GAAAATGGAA GGTAAGGTTG TGC

Primers were based on the sequence given in the Genbank DNA sequence entry ATAGAMSG for the genomic *agamous* sequence. Primer 1 contains an added HindIII restriction site at its 5' end. Primer 2 contains an added BamHI restriction site at its 5' end.

The following primers were designed from the Genbank sequence entry ATHAPETALA in order to amplify out part of the *apetala3* gene sequence:

Primer 3 (SEQ.ID. No.3)

ATCGGGATCC ATGGGCTCAC GGTTTTGTGT GA

Primer 4 (SEQ.ID. No.4)

ATCGGAGCTC TTATTCAAGA AGATGGAAGG TAATGA

Primer 3 was specifically designed to begin amplification at position 992 of the published *apetala3* sequence, which is a start codon in the wrong reading frame, to produce an active product and which avoids the initial part of the sequence that has strong homologies with other MADS box genes in the same gene family. Primer 3 also contained a one base pair change from the published sequence to remove an unwanted SacI restriction site. Primer 3 has a BamHI restriction site at its 5' end. Primer 4 has a SacI site at its 5' end. A 1586 bp fragment was amplified from *Arabidopsis* DNA using primers 3 & 4 using PCR.

The following cloning methods are familiar to any one skilled in the art and the results can be obtained by following the methods in Sambrook *et al* (1989). The *agamous* promoter fragment was ligated into a pBluescript derived vector (Stratagene Ltd., Cambridge, UK) as a HindIII-BamHI fragment. The altered *apetala* gene fragment was ligated downstream of the *agamous* promoter as a BamHI-SacI fragment in the same vector. The vector also contained a nos terminator sequence downstream of the altered *apetala3* sequence as a SacI-EcoRI fragment. The vector was named pDVM37010. A second plasmid containing the

agamous promoter in front of the m-gfp-ER reporter gene obtained from Jim Haselhoff, MRC was made as a control and was named pDVM37003.

The promoter-gene fragment-terminator cassettes were excised from pDVM37010 (SEQ.ID. No.5) and pDVM37003 (SEQ.ID. No. 6) as NotI restriction fragments and ligated into a pBIN19 (Bevan, M. (1984)) derived vector pATC to give plasmids pATC37010 (map shown in Figure 3) and pATC37003 (map shown in Figure 4). These sequences could be cloned into any other equivalent vector which has suitable restriction sites therein, i.e. NotI at each end of the cassette. The map for pATC is shown in Figure 5. It has modified restriction sites compared with PBIN19. pATC37010 produces a co-suppression product under the control of the *agamous* promoter to inactivate *apetala3* function in the developing stamens of the flower.

The plasmids were transferred into *Agrobacterium tumefaciens* host LBA4404 and used to transform *Arabidopsis thaliana* following the method of Bechtold *et al* (1993) and Nicotiana tabacum *cv K326 using the method of* Horsch *et al* (1985). 8 *Arabidopsis* plantlets transgenic for pATC37010 and 6 *Arabidopsis* plantlets transgenic for pATC37003 were obtained. Three sets of one hundred leaf discs were used for *Nicotiana* transformation for each construct. Transgenic callus growth was detected for all three sets.

The *Arabidopsis* plantlets were transferred into soil 10-14 days after germination and grown until flowering. The flowers exhibited no stamens, and double rose carpels.

The kanamycin resistant pATC37010 transgenic plants were further screened for the presence of the desired inserts by PCR with Taq polymerase using primers 1 and 4 following procedures known to one skilled in the art. The PCR was conducted for 40 cycles of incubation involving the steps of incubation at 94°C for 60 seconds, 60°C for 30 seconds and 72°C for 140 seconds. PCR positive samples were identified by visualisation of the PCR products upon agarose gel electrophoresis following procedures known to one skilled in the art.

The control pATC37003 transgenic plants were further screened for the presence of the desired inserts by PCR with Taq polymerase using primers 1 and primer 7 following procedures known to one skilled in the art. The PCR was conducted for 40 cycles of incubation involving the steps of incubation at 94°C for 40 seconds, 60°C for 30 seconds and 72°C for 140 seconds. PCR positive samples were identified by visualisation of the PCR products upon agarose gel electrophoresis following procedures known to one skilled in the art.

Primer 7 (SEQ. ID No. 7)

GAACTGGGAC CACTCCAGTG

In both cas s, transgenic plants containing the appropriate construct were identified.

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SEQUENCE LISTING

(1) GENERAL INFORMATION

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(ii) TITLE OF INVENTION: Improvements Relating to the Specificity of Gene Expression

(iii) NUMBER OF SEQUENCES: 6

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:	Diskette 3.50 inch
(B) COMPUTER:	Viglen P5/75
(C) OPERATING SYSTEM:	MS-DOS Windows 3.1
(D) SOFTWARE:	Microsoft Word 2.0

(vi) CURRENT APPLICATION DATA:

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(C) CLASSIFICATION:	Not yet known

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(viii) ATTORNEY/AGENT INFORMATION:

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(2) INFORMATION FOR SEQ. ID. NO:1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	32
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) MOLECULE TYPE: synthetic primer

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE:

(A) NAME:	Hind III restriction site
(B) LOCATION:	5-10

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO:1:

ATCGAAGCTT CTAAATGTAC TGAAAAGAAA CA 32

(2) INFORMATION FOR SEQ. ID. NO:2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic primer

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(iv) ANTISENSE: yes

(ix) FEATURE:
(A) NAME: Bam HI restriction site
(B) LOCATION: 5-10

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO:2:

ACTGGGATCC GAAAATGGAA GGTAAGGTTG TGC 33

(2) INFORMATION FOR SEQ. ID. NO:3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32
(B) TYPE nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic primer

(ix) FEATURE:
(A) NAME: Bam HI restriction site
(B) LOCATION: 5-10

(ix) FEATURE:
(A) NAME: start codon
(B) LOCATION: 11-13

(ix) FEATURE:
(A) NAME: deliberate base change from A to G
(B) LOCATION: 14

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO: 3:

ATCGGGATCC ATGGGCTCAC GGTTTTGTGT GA 32

(2) INFORMATION FOR SEQ. ID. NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	36
(B) TYPE	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) MOLECULE TYPE: synthetic primer

(iv) ANTISENSE: yes

(ix) FEATURE:

(A) NAME:	Sac I restriction site
(B) LOCATION:	5-10

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO: 4:

ATCGGAGCTC TTATTCAAGA AGATGGAAGG TAATGA 36

(2) INFORMATION FOR SEQ. ID. NO:5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	2319
(B) TYPE	nucleic acid
(C) STRANDEDNESS:	double
(D) TOPOLOGY:	linear in source circular in plasmid

(ii) MOLECULE TYPE: plasmid DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM:	Arabidopsis thaliana
(B) STRAIN:	Landsberg

(ix) FEATURE:

(A) NAME/KEY:	promoter - Arabidopsis agamous (Genbank ATAGAMSG)
(B) LOCATION:	26-441

(ix) FEATURE:

(A) NAME/KEY:	coding sequence - Arabidopsis apetala3 (Genbank ATHPETALA)
(B) LOCATION:	448-2013

(ix) FEATURE:

(A) NAME/KEY:	Nos terminator
(B) LOCATION:	2020-2286

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO: 5:

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TCAACTTTCA CCACATAATT ATCTAACATG TGTATGTTCC AAAACCAGTT TAAATGAATT     180
ACTTTTCAGA AAATACATGT ATATTAACTC TATCTAATAA AGAAGAAACA CATACTTATC     240
TCATAGATTC CATTCATAAA ACTATGCTTT AGTGAGTAAG AAAACCAGTA ATCAAACACA     300
AATTGACAAG ACACTATATG GATGTAAAAA GTGGGGAAAA TATGGTGATA AATAGTAGAG     360
AAAATTAAAA AGAAAAAATA ATATTCCTTT ATAAATGTAT ATACCCATCT CTTCACCAGC     420
ACAACCTTAC CTTCCATTTT CGGATCCATG GGCTCACGGT TTTGTGTGAT GCTAGGGTTT     480
CGATTATCAT GTTCTCTAGC TCCAACAAGC TTCATGAGTA TATCAGCCCT AACACCACGT     540
ACACCATCTC TCTAAACACC ACTCTTAAAT TAAGCTAATT GAGTTGCTTT GTTTTCTTAT     600
AATTAACCAC TACTTTTTTG GTGATTTTGT TGGTTATAGA ACGAAGGAGA TCGTAGATCT     660
GTACCAAACT ATTTCTGATG TCGATGTTTG GGCCACTCAA TATGAGGTTT TTTTCCTTCT     720
TAGATCTTTC TTCTTCTTCT TGATATGTGT TTCGCTGGTT GGTTAAATTC TTGATGCGTT     780
TTGCTGCAGC GAATGCAAGA AACCAAGAGG AAACTGTTGG AGACAAATAG AAATCTCCGG     840
```

ACTCAGATCA AGTATTTGTT TCTTCTCTCT TCTCTTAGAT GAGGAGTTTT ACTAAAAAAA 900

ATGAGTACGG AAATATACAT ATTTTTAAAA TTGTAGGCAG AGGCTAGGTG AGTGTTTGGA 960

CGAGCTTGAC ATTCAGGAGC TGCGTCGTCT TGAGGATGAA ATGGAAAACA CTTTCAAACT 1020

CGTTCGCGAG CGCAAGGTTC TCTTCATACT TTTCCCTTAC CTAGGGTTTC AATTAATCCT 1080

ATATACCCAA GCTTCAGTTT TGAATTGAAT TATTAAAAAA TGAATTTTAT TGTTGTATAT 1140

ATGTTTTAGA AAAAGAAACA TTTTGTTTAC TGTTGGATAA TATATGTTAA TTGTATTGTA 1200

CTGTACAGTT CAAATCTCTT GGGAATCAGA TCGAGACCAC CAAGAAAAAG GTCACATCTT 1260

CTATGTCCAC TCACTTTTCC ATTTTATCAT ATTTATTTGT CTCAACAATT TTGTGACAAT 1320

TGAATTTATC AACTTACTAA AACTGTTGAT AACACTTTTC TTGGACAATT ATATTTGTGT 1380

GTGTGTGTGT GTGTGTTTAA GCTAATGGAT AAAGAAAATA CCAAGTATAC TATATAGTGA 1440

TGTCATAATA ACTTGGGTAT ATATCTTCAT AATTTTTTTG GGTGGGAATA TTTCTTCATA 1500

ATTTCTCTTG TGGTTTACAC AATTGCAGAA CAAAAGTCAA CAGGACATAC AAAAGAATCT 1560

CATACATGAG CTGGTAATAT CTCTTTCTGT TTTTCTCAAA TGTTGGTTTA GGCATAATAC 1620

ATTCATGGAA TACGGAGCCA GTTAAAAAGA TATCTAGAAA TGTAGTGTAG ATTGATCAGT 1680

CACTCTTATG TTTTCTTGTG ATTCTCTTAT CGAAATATCT CCTAGTTAAA TCATATATCA 1740

AATGTCATGT CATTTCGAAT TAATAATATT GGTTTTAGTT ATGTGGAATA TGGCTTAAAA 1800

CATGTTTTGG TGAATTAGGA ACTAAGAGCT GAAGATCCTC ACTATGGACT AGTAGACAAT 1860

GGAGGAGATT ACGACTCAGT TCTTGGATAC CAAATCGAAG GGTCACGTCG TTACGCTCTT 1920

CGTTTCCACC AGAACCATCA CCACTATTAC CCCAACCATG GCCTTCATGC ACCCTCTGCC 1980

TCTGACATCA TTACCTTCCA TCTTCTTGAA TAAGAGCTCG AATTTCACCC GATCGTTCAA 2040

ACATTTGGCA ATAAAGTTTC TTAAGATTGA ATCCTGTTGC CGGTCTTGCG ATGATTATCA 2100

TATAATTTCT GTTGAATTAC GTTAAGCATG TAATAATTAA CATGTAATGC ATGACGTTAT 2160

TTATGAGATG GGTTTTTATG ATTAGAGTCC CGCAATTATA CATTTAATAC GCGATAGAAA 2220

ACAAAATTAT GCGCGCAAAC TAGGATAAAT TATCGCGCGC GGTGTCATCT ATGTTACTAG 2280

ATCGGGAATT CTGTTTAAAC TCGAGACTAG TGCGGCCGC 2319

(2) INFORMATION FOR SEQ. ID. NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1559
(B) TYPE	nucleic acid
(C) STRANDEDNESS:	double
(D) TOPOLOGY:	linear in source circular in plasmid

(ii) MOLECULE TYPE: plasmid DNA

(ix) FEATURE:

(A) NAME/KEY:	promoter - Arabidopsis agamous (Genbank ATAGAMSG)
(B) LOCATION:	26-441

(ix) FEATURE:

(A) NAME/KEY:	coding sequence - green fluorescent protein
(B) LOCATION:	443-1258

(ix) FEATURE:

(A) NAME/KEY:	Nos terminator
(B) LOCATION:	1260-1526

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO: 6:

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TCAACTTTCA CCACATAATT ATCTAACATG TGTATGTTCC AAAACCAGTT TAAATGAATT     180
ACTTTTCAGA AAATACATGT ATATTAACTC TATCTAATAA AGAAGAAACA CATACTTATC     240
TCATAGATTC CATTCATAAA ACTATGCTTT AGTGAGTAAG AAAACCAGTA ATCAAACACA     300
AATTGACAAG ACACTATATG GATGTAAAAA GTGGGGAAAA TATGGTGATA AATAGTAGAG     360
AAAATTAAAA AGAAAAAATA ATATTCCTTT ATAAATGTAT ATACCCATCT CTTCACCAGC     420
ACAACCTTAC CTTCCATTTT CGGATCCAAG GAGATATAAC AATGAAGACT AATCTTTTTC     480
TCTTTCTCAT CTTTTCACTT CTCCTATCAT TATCCTCGGC CGAATTCAGT AAAGGAGAAG     540
AACTTTTCAC TGGAGTTGTC CCAATTCTTG TTGAATTAGA TGGTGATGTT AATGGGCACA     600
AATTTTCTGT CAGTGGAGAG GGTGAAGGTG ATGCAACATA CGGAAAACTT ACCCTTAAAT     660
TTATTTGCAC TACTGGAAAA CTACCTGTTC CATGGCCAAC ACTTGTCACT ACTTTCTCTT     720
ATGGTGTTCA ATGCTTTTCA AGATACCCAG ATCATATGAA GCGGCACGAC TTCTTCAAGA     780
GCGCCATGCC TGAGGGATAC GTGCAGGAGA GGACCATCTT CTTCAAGGAC GACGGGAACT     840
ACAAGACACG TGCTGAAGTC AAGTTTGAGG GAGACACCCT CGTCAACAGG ATCGAGCTTA     900
AGGGAATCGA TTTCAAGGAG GACGGAAACA TCCTCGGCCA CAAGTTGGAA TACAACTACA     960
ACTCCCACAA CGTATACATC ATGGCCGACA AGCAAAAGAA CGGCATCAAA GCCAACTTCA    1020
AGACCCGCCA CAACATCGAA GACGGCGGCG TGCAACTAGC TGATCATTAT CAACAAAATA    1080
```

CTCCAATTGG CGATGGCCCT GTCCTTTTAC CAGACAACCA TTACCTGTCC ACACAATCTG 1140

CCCTTTCGAA AGATCCCAAC GAAAAGAGAG ACCACATGGT CCTTCTTGAG TTTGTAACAG 1200

CTGCTGGGAT TACACATGGC ATGGATGAAC TATACAAACA TGATGAGCTT TAAGAGCTCG 1260

AATTTCACCC GATCGTTCAA ACATTTGGCA ATAAAGTTTC TTAAGATTGA ATCCTGTTGC 1320

CGGTCTTGCG ATGATTATCA TATAATTTCT GTTGAATTAC GTTAAGCATG TAATAATTAA 1380

CATGTAATGC ATGACGTTAT TTATGAGATG GGTTTTTATG ATTAGAGTCC CGCAATTATA 1440

CATTTAATAC GCGATAGAAA ACAAAATTAT GCGCGCAAAC TAGGATAAAT TATCGCGCGC 1500

GGTGTCATCT ATGTTACTAG ATCGGGAATT CTGTTTAAAC TCGAGACTAG TGCGGCCGC 1559

(2) INFORMATION FOR SEQ. ID. NO:7

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic primer

(iii) ORIGINAL SOURCE
(A) ORGANISM: Aequorea victoria

(iv) ANTISENSE yes

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO: 7

GAACTGGGAC CACTCCAGTG 20

Applicant's or agent's file reference number RD-ATC-17	International Application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 11, line 27

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections for Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St. Machar Drive
Aberdeen AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
20/03/97	NCIMB 40870

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on additional sheet ☐

- *Agrobacterium tumefaciens* Strain Number C58 pBIN 05101
- Please find enclosed a copy of the Receipt of Deposit and a copy of the viability proof from the Depositry Institution.
- Name and address of Depositor: Advanced Technologies (Cambridge) Limited, 210 Cambridge Science Park, Cambridge CB4 4WA, United Kingdom.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leaf blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only

☐ This sheet was received with the international application

21 APRIL 1998

Authorized officer

T Moscos

For receiving Office use only

☐ This sheet was received by the international Bureau on:

Authorized officer

Form PG/RO/134 (July 1992)

Advanced Technologies
(Cambridge) Ltd.,
210 Cambridge Science Park,
Cambridge.
CB4 4WA

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: Agrobacterium tumefaciens C58 pBIN05101	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40870

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 20 March 1997 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on . (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd

Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 31 March 1997 Terence Dando

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

Advanced Technologies
(Cambridge) Ltd.,
210 Cambridge Science Park,
Cambridge.
CB4 4WA

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Agrobacterium tumefaciens C58 pBIN05101	NCIMB 40870

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a.scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 20 March 1997(date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on . (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd
Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 31 March 1997 Terence Dando

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Advanced Technology (Cambridge) Ltd.,
210 Cambridge Science Park,
Cambridge.
CB4 4WA

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AS ABOVE Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40870 Date of the deposit or of the transfer: 20 March 1997
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 26 March 1997 [2]. On that date, the said microorganism was [X] [3] viable [ ] [3] no longer viable	

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

Form BP/9 (first page)

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd

Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Terence Dando

Date: 31 March 1997

[4] Fill in if the information has been requested and if the results of the test were negative.

Form BP/9 (second and last page)

Applicant's or agent's file reference number RD-ATC-17	International Application No

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 11, line 6

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections for Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St. Machar Drive
Aberdeen AB2 1RY
Scotland, United Kingdom

Date of deposit	Accession Number
20/03/97	NCIMB 40871

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on additional sheet ☐

- *Agrobacterium tumefaciens* Strain Number C58 pBIN 05002
- Please find enclosed a copy of the Receipt of Deposit and a copy of the viability proof from the Depositry Institution.
- Name and address of Depositor: Advanced Technologies (Cambridge) Limited, 210 Cambridge Science Park, Cambridge CB4 4WA, United Kingdom.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leaf blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For receiving Office use only
☐ This sheet was received with the international application	☐ This sheet was received by the international Bureau on:
21 APRIL 1998	
Authorized officer	Authorized officer
T Hudson	

Form PG/RO/134 (July 1992)

Advanced Technologies
(Cambridge) Ltd.,
210 Cambridge Science Park,
Cambridge.
CB4 4WA

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Agrobacterium tumefaciens C58 pBIN05002	NCIMB 40871

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 20 March 1997 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd

Address: 23 St Machar Drive, Aberdeen Scotland UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Terence Dando

Date: 31 March 1997

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Advanced Technology (Cambridge) Ltd.,
210 Cambridge Science Park,
Cambridge.
CB4 4WA

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AS ABOVE Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40871 Date of the deposit or of the transfer: 20 March 1997
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 26 March 1997 [2]. On that date, the said microorganism was [3] [X] viable [3] [ ] no longer viable	

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

Form BP/9 (first page)

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd

Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Terence Dando

Date: 31 March 1997

[4] Fill in if the information has been requested and if the results of the test were negative.

CLAIMS

1. A method of improving the specificity of gene regulation in a transformed organism, the method comprising the steps of identifying an endogenous target gene in an organism, determining the location of more than one expression sites of the target gene, creating a chimaeric gene comprising a promoter which causes gene expression at at least two expression sites in an organism, including expression at one of the expression sites of the target gene, and an agent being a nucleic acid sequence which regulates expression of the target gene or a product thereof, stably incorporating the chimaeric gene into a cell of the organism by genetic transformation, and regenerating an organism from the transformed cell, which transformed organism contains the chimaeric gene, the expression of which gene in the organism causes the target gene or a product thereof to be regulated at at least one specific expression site in the transformed organism or progeny thereof.
2. A method according to Claim 1, wherein said nucleic acid sequence is a coding or a non-coding sequence.
3. A method according to Claim 1 or 2, wherein the expression of said target gene is up regulated.
4. A method according to Claim 1 or 2, wherein the expression of said target gene is down regulated.
5. A method according to Claims 1, 2, 3 or 4, wherein said organism is a plant.

6. A method according to any one of Claims 1 to 4, wherein said nucleic acid sequence is in antisense orientation.

7. A method according to Claim 1, 2 or 4, wherein said nucleic acid is in sense orientation.

8. A method according to Claim 1, 2 or 3, wherein said nucleic acid is in sense orientation.

9. A method according to Claim 3, wherein up regulation of said target gene occurs from the introduction of an activator of the promoter of said target gene.

10. A method according to any one of the preceding claims, wherein said agent of said chimaeric gene comprises one or more nucleic acid sequences, each of which sequences, when expressed, carries out a particular function.

11. A method according to any one of the preceding claims, wherein the promoter of said chimaeric gene is expressed in more than one overlapping expression site of said endogenous gene.

12. A method according to any one of the preceding claims, wherein said promoter is the KNT1 promoter or the RB7 promoter.

13. A method according to any one of Claims 1-11, wherein said promoter is caused to express by other agents acting on them.

14. A method according to Claim 1-12, wherein said nucleic acid sequence is the or a part of the antisense sequence of the RB7 or KNT1 promot r or coding sequence thereof.

15. A method according to any one of the Claims 1-11, wherein said nucleic acid sequence is one or more of a ribozyme, a

targeted RNase to degrade messeng r RNA, or a stabilising or destabilising agent of a specific RNA.

16. A method according to any one of Claims 1-11 and 13, wherein said nucleic acid comprises an activator of transcription.

17. A method according to Claim 16, wherein said activator of transcription is a heat shock factor encoding a protein.

18. A method according to any one of the preceding claims, wherein the mechanism whereby said agent acts on said gene is any one or more of said mechanisms numbered 1-11 hereinabove.

19. A construct deposited under accession number NCIMB 40871.

20. A construct comprising the plasmid pATC 37010 or a promoter-gene fragment-terminator cassette such as that contained in that plasmid.

21. Plants and propagules thereof transformed by the method according to any one of Claims 1-18.

Fig.1.

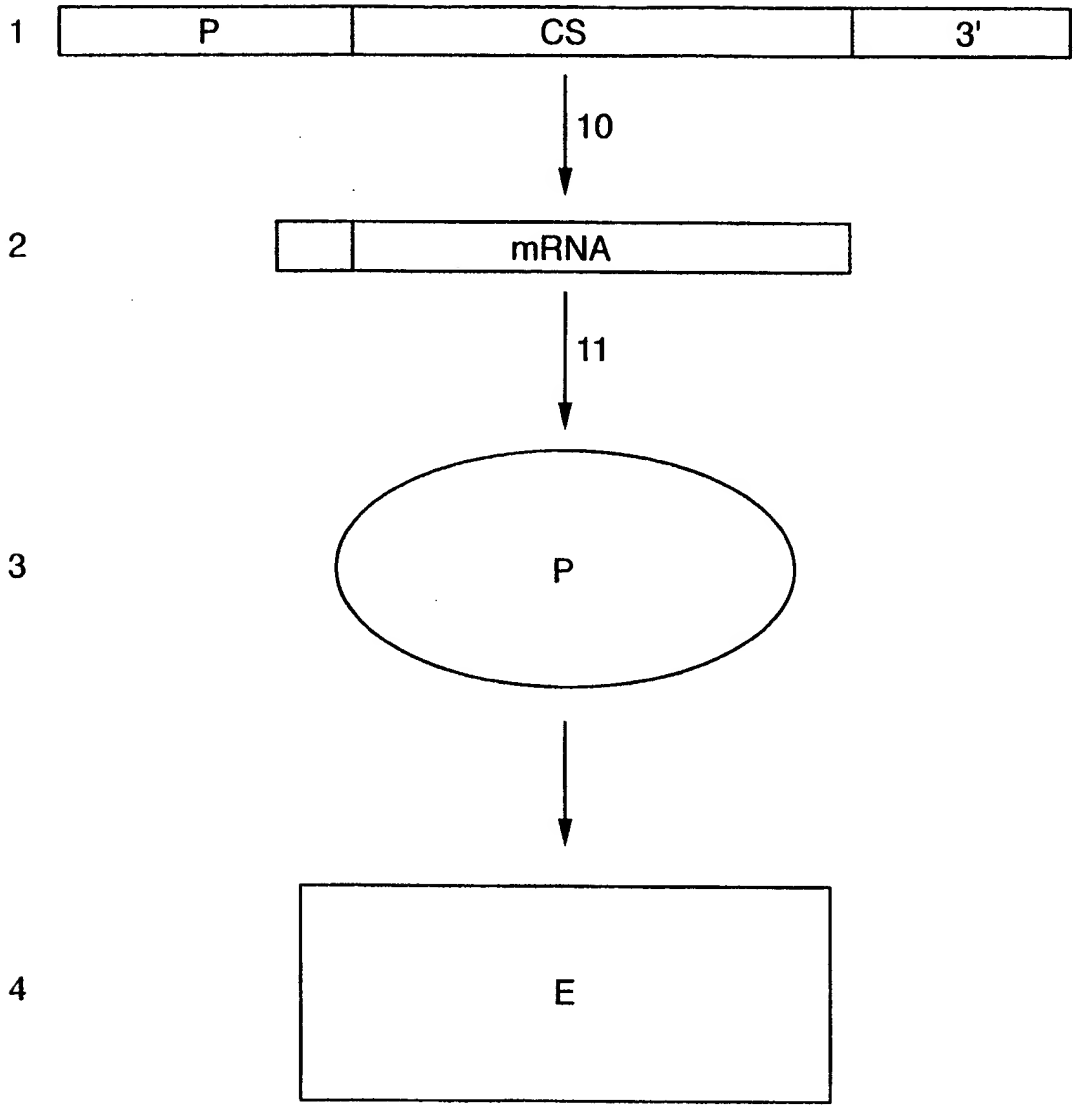


Fig.2.

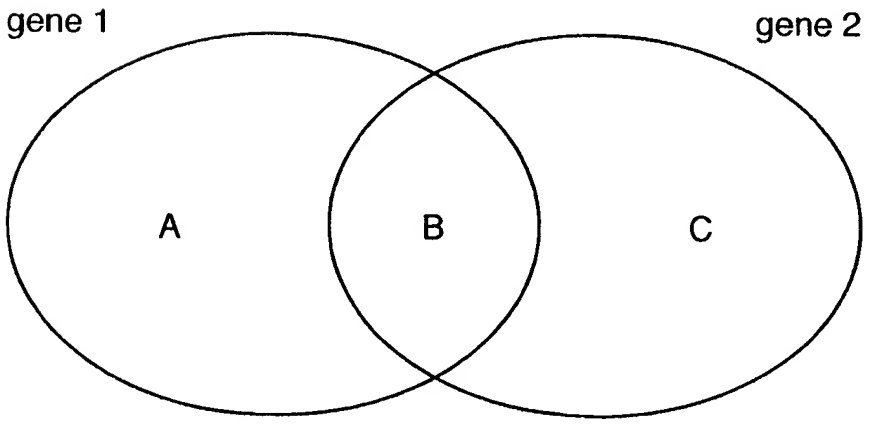
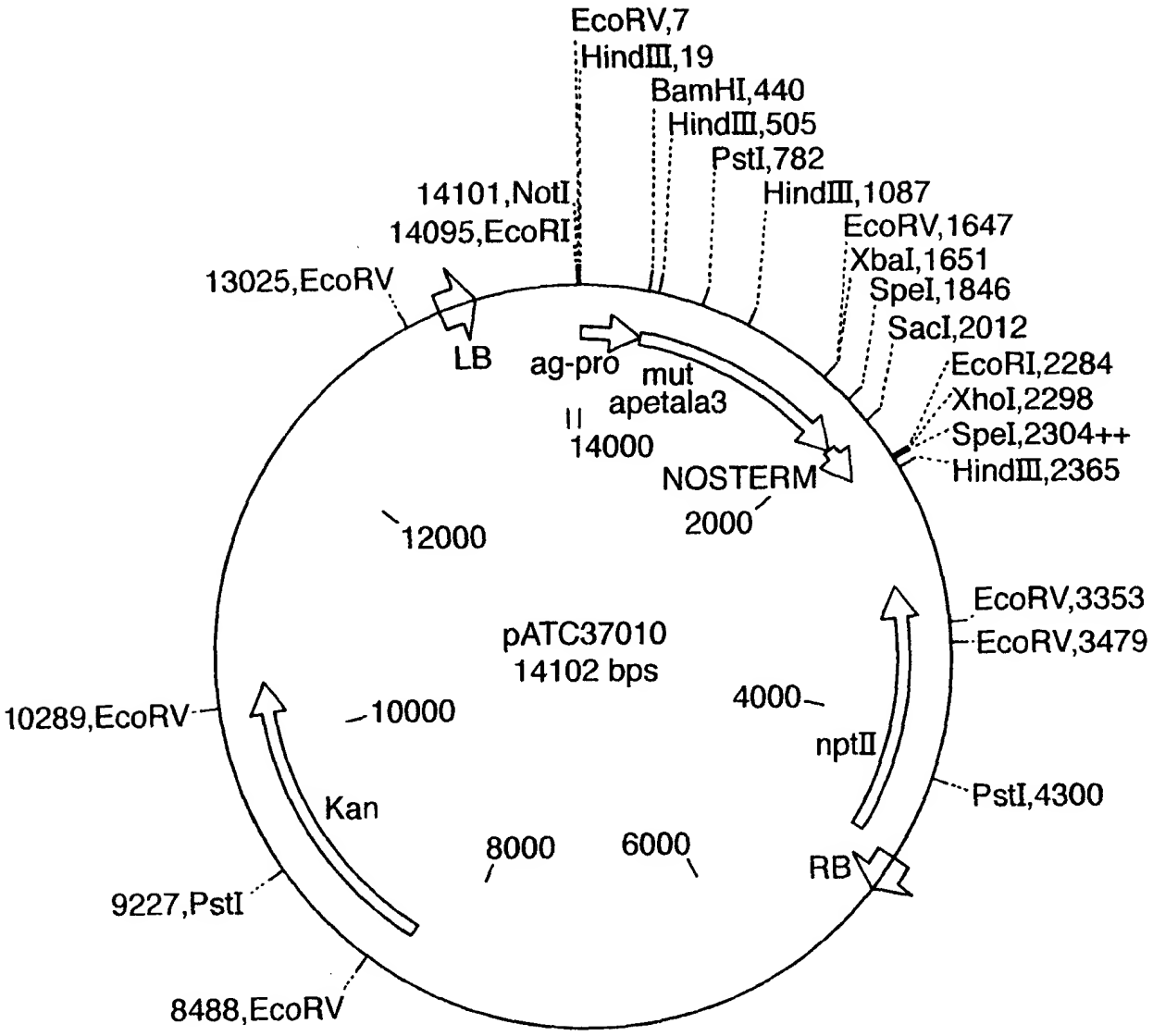


Fig.3.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SpeI,1846
SacI,2012
EcoRI,2284
XhoI,2298
SpeI,2304++
HindIII,2365
14101,NotI
14095,EcoRI
13025,EcoRV
LB
ag-pro
mut
apetala3
14000
NOSTERM
2000
12000
EcoRV,3353
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pATC37010
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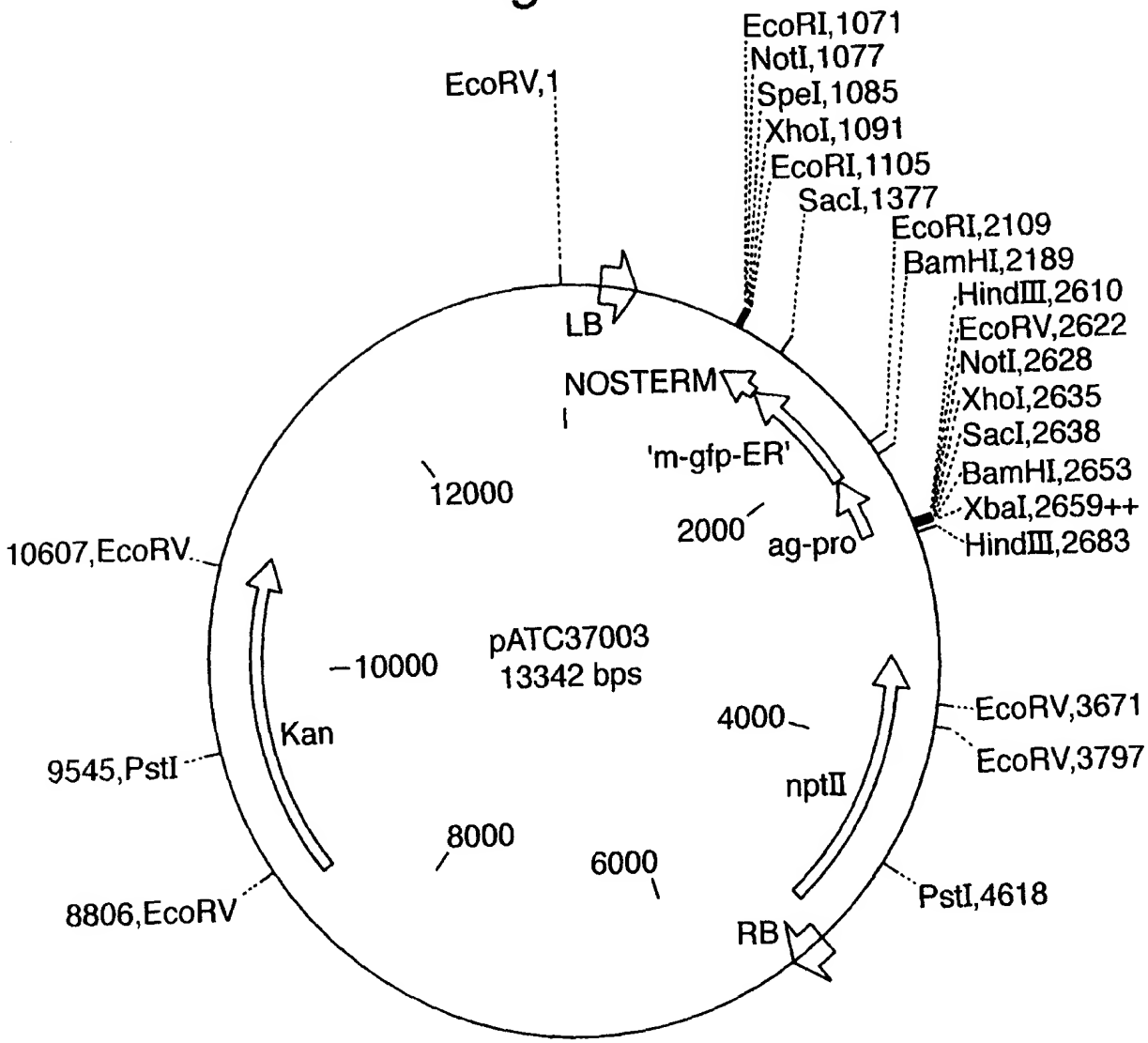
8488,EcoRV

Fig.4.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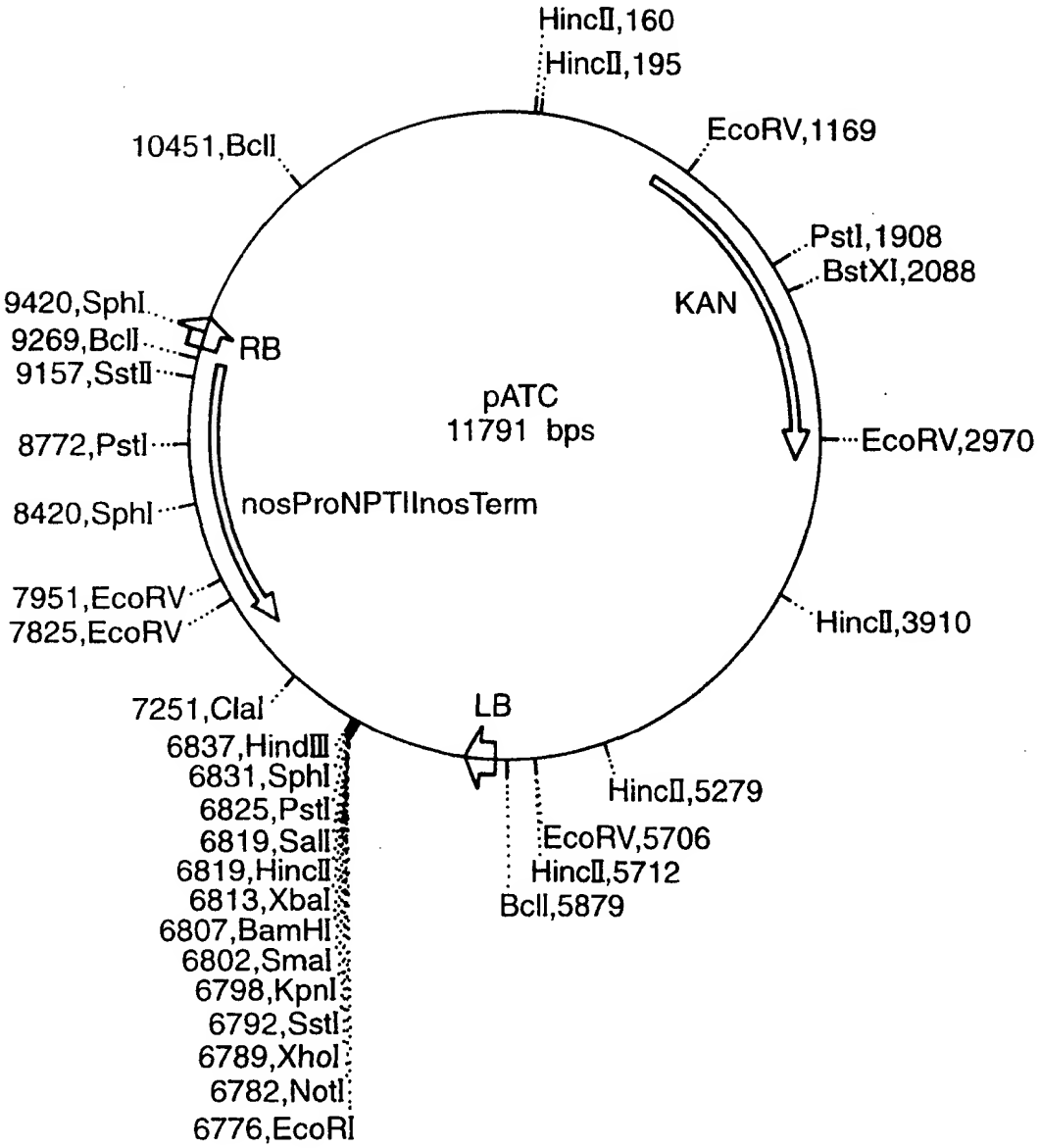
NotI,1077
SpeI,1085
XhoI,1091
EcoRI,1105
SacI,1377
EcoRI,2109
BamHI,2189
HindIII,2610
EcoRV,2622
NotI,2628
XhoI,2635
SacI,2638
BamHI,2653
XbaI,2659++
HindIII,2683
EcoRV,1
LB
NOSTERM
'm-gfp-ER'
ag-pro
12000
2000
10607,EcoRV
pATC37003
13342 bps
10000
Kan
9545,PstI
4000
nptII
EcoRV,3671
EcoRV,3797
8000
6000
8806,EcoRV
RB
PstI,4618

Fig.5.


HincII,160
HincII,195
EcoRV,1169
PstI,1908
BstXI,2088
KAN
EcoRV,2970
HincII,3910
HincII,5279
EcoRV,5706
HincII,5712
BclI,5879
LB
7251,ClaI
6837,HindIII
6831,SphI
6825,PstI
6819,SalI
6819,HincII
6813,XbaI
6807,BamHI
6802,SmaI
6798,KpnI
6792,SstI
6789,XhoI
6782,NotI
6776,EcoRI
7951,EcoRV
7825,EcoRV
8420,SphI
8772,PstI
9157,SstII
9269,BclI
9420,SphI
RB
10451,BclI
pATC
11791 bps
nosProNPTIInosTerm